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CONTAMINATION OF LETTUCE WITH ANTIBIOTIC RESISTANT *E. COLI* AFTER SLURRY APPLICATION

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Due to disease outbreaks associated with contaminated vegetables it has been speculated to what extent this may be linked with application of animal manure as fertilizer, which is particularly practiced in organic vegetable production where conventional fertilizers are prohibited. A field survey was therefore performed to assess the survival and transfer of antibiotic-resistant *E. coli* from animal manure to lettuces, with *E. coli* serving as an indicator of bacterial enteric pathogens.

Animal slurry was applied to 3 Danish fields prior to planting of lettuce seedlings, then 5-8 weeks later at the normal time of harvest, inner and outer leafs of 10 lettuce heads were pooled into one sample unit with a total of 50 pools per field. Additionally, in one field, 15 soil samples were collected weekly until the harvest time. *E. coli* was enumerated by plating 1 mL of 10-fold serial dilutions of 5 g of homogenized sample material, i.e. manure, soil and lettuce onto PetrifilmTM Select *E. coli* count plates (3M) containing 16 mg/L streptomycin or 16 mg/L ampicilin or no antibiotics. Plates were then incubated 24 h at 44°C. Selected isolates of *E. coli* (n=83) from slurry, soil and lettuce were analysed by PFGE DNA typing for further discrimination.

The slurry applied to the fields contained 3.0-4.5 Log10 *E. coli* CFU/g and resistant *E. coli* ranged from 1.0 to 4.4 Log10 *E. coli* CFU/g with particular high numbers of streptomycin resistant *E. coli* in conventional pig slurry (field 1) opposed to organic cow slurry (field 2 and 3). *E. coli* was found in 36-54% of the pooled lettuce samples at the three fields with a detection limit of 10 CFU/g and 10-18% and 0-2% of pools had streptomycin and ampicilin resistant *E. coli,* respectively. Unexpectedly, the highest percentage of lettuce pools with antibiotic resistant *E. coli* were found on fields fertilized with organic cow slurry where 0.1-5% of *E. coli* was resistant opposed to 5-50% resistant *E*. *coli* in conventional slurry. Numbers of *E. coli* in 14-20% of pooled lettuce samples exceeded a satisfactory microbiological hygiene criteria level of 100 CFU/g. The numbers of resistant *E. coli* for both antibiotics were approximately 10-fold lower than the sensitive *E. coli.*

At the time of harvest, the numbers of *E. coli* in 5 of 15 soil samples were reduced below the detection limit and no samples exceeded 100 CFU/g, which was in contrast to the lettuce samples, where 20% of faecally contaminated samples contained >100 *E. coli*/g. This indicates that fecal contamination of crops originated from alternative sources such as contaminated water or wildlife. This was supported by genotyping of *E. coli,* where half of the 21 PFGE types were found on single occasions in either soil or lettuce, whereas the other half was found both in slurry and lettuce indicating a possible transfer.

Key words: lettuce, manure fertilizer, faeces contamination, resistant bacteria, food safety

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